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Engineering the CNS stem cell microenvironment

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Abstract

The loss of neural tissue underlies the symptomatology of several neurological insults of disparate etiology, including trauma, cerebrovascular insult and neurodegenerative disease. Restoration of damaged neural tissue through the use of exogenous or endogenous neural stem or progenitor cells is an enticing therapeutic option provided one can control their proliferation, migration and differentiation. Initial attempts at CNS tissue engineering relied on the intrinsic cellular properties of progenitor cells; however, it is now appreciated that the microenvironment surrounding the cells plays an indispensable role in regulating stem cell behavior. This article focuses on attempts to engineer the neural stem cell microenvironment by utilizing the major cellular components of the niche (endothelial cells, astrocytes and ependymal cells) and the extracellular matrix in which they are embedded.

Keywords

microenvironment; neural progenitor cell; neural stem cell; neural stem cell niche

Neural stem cells (NSCs) are defined by their capacity to self-renew indefinitely and to produce all cell types of the mature CNS. In the adult brain, NSCs are largely restricted to two regions: the subventricular zone (SVZ), which is located along the lateral wall of the lateral ventricle; and the subgranular zone (SGZ), which is located between the dentate gyrus and the hilus of the hippocampus [1–3]. The SVZ supplies granular and periglomerular neurons to the olfactory bulb, via the rostral migratory stream, while the SGZ produces local granule neurons for the dentate gyrus. This regional localization of NSCs is partially directed by the SVZ and SGZ microenvironments, or niches, surrounding the cells.

The influence of the microenvironment is apparent in studies where proliferating cells from non-neurogenic areas of the CNS are induced to self-renew and produce differentiated progeny. This can be accomplished either by providing niche signals to the normally non-neurogenic cells or by transplanting these cells into neurogenic areas of the brain [4–6]. With transplantation, the type of progeny produced is dependent on the location of transplantation [7–9]. Conversely, the neurogenic potential of NSCs that are grafted into a non-neurogenic area is severely limited [7–9]. This lends credence to the idea that a stem cell should be thought of as a state rather than an entity [10] and further suggests that this state is controlled in large

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part by the microenvironment. Intrinsic cell properties, however, do play a major role in the SVZ in determining which olfactory bulb neuronal subtype is ultimately produced. Regional and age-associated differences in SVZ NSC fate persist despite varying *ex vivo* culture conditions or heterotypic transplantation [11,12]. Thus, the source of NSCs for expansion and transplantation should also be carefully considered as well as the method of isolation. It should be noted that the studies discussed here utilize a variety of model systems with cells derived using different methodologies [1]. For the purposes of this review, the term neural progenitor cell (NPC) will be used as a general term encompassing both NSCs and their surrounding heterogeneous progenitor cells, which are often also isolated.

These microenvironments serve a critical role in both NPC maintenance and activation. In response to physiological and pathological changes, the adult brain is capable of activating NPCs within these regions, increasing their rate of self-renewal and neurogenesis. During exercise, hippocampal neurogenesis increases, leading to antidepressive effects and improvements in memory and learning [13–16]. During stroke or other hypoxic insults, the proliferation of NPCs in the SVZ increases and these newly born cells can migrate to the site of injury and mitigate damage [17–19]. The signals emanating from the niche that controls this activation are just beginning to be elucidated.

While the activation of NPCs in the adult brain is exciting, it does not always occur, and when it does occur it is often not able to fully compensate for the damage. Therapeutically, to enhance neurogenesis in the adult brain, either exogenous NPCs can be transplanted or endogenous NPCs can be further activated. Early efforts have focused on exogenous NPC transplantation with little attention being paid to the host or transplantation microenvironments. Given changes that occur in the microenvironment during disease, NPCs cannot be expected to behave normally without their native instructions, which could be provided by a transplantation microenvironment. Engineering NPC microenvironments will facilitate several branches of regenerative medicine including: *ex vivo* expansion and differentiation of NPCs for transplantation, identification of targets for activating endogenous NPCs, generation of scaffolds to direct behavior of exogenous and/or endogenous NPCs, and creation of physiologically relevant *in vitro* cell culture platforms to study the basic biology of NPCs. This article will focus on engineering efforts to mimic various components of the NPC niche with the aim of restoring damaged neural tissue. We will focus on the major cellular components of the niche – endothelial cells, astrocytes and ependymal cells – and the extracellular matrix (ECM) in which they are embedded.

The NSC niche

The NSC niche serves as an anatomical and functional compartment for NPCs. These niches contain many of the conserved components found in other stem cell niches, namely the resident stem cells, vessels, stromal cells and a specialized ECM [20–22]. Functionally, within the niche, stem cell behavior is controlled by an integration of local signals produced by niche components and distant signals carried to the niche via the vasculature and/or neural inputs. While distant signals do play a significant role in stem cell biology [23], this article will focus on the local signals produced by niche components. Understanding the basic biology of NPCs and their niche components has laid the groundwork for utilization of both components in engineering applications.

The SVZ contains SVZ astrocytes (which include populations of stem cells and support cells), immature precursors, neuroblasts, ependymal cells and endothelial cells (ECs) [24], as illustrated in Figure 1. The resident stem cell lineage in the SVZ consists of the relatively quiescent NPCs (type B cells), which can self-renew or give rise to rapidly dividing transit-amplifying cells (type C cells). The type C cells ultimately give rise to migratory neuroblasts

(type A cells), which enter the rostral migratory stream [2]. A group of recent studies has helped to elucidate the ultrastructural organization of the SVZ niche. A layer of ependymal cells lines the ventricle and is penetrated by the apical process of an NPC with a short single cilium at sites of adult neurogenesis [25]. Beneath this layer the bodies of type B cells are organized into chains of tunnels through which type A cells migrate. Proliferative clusters of type C cells reside along the chains of type A cells. Within this layer is an extensive network of blood vessels that run along and within type A cell chains. The basal processes of NPCs also contact this vasculature [26]. At these sites of contact, a modified blood–brain barrier exists, which lacks astrocytic endfeet and pericytic coverage [27]. The vessels also produce a laminin rich extravascular basal lamina, which is organized into branched structures known as fractones [28].

The SGZ contains SGZ astrocytes, dividing immature cells, neuroblasts and ECs [29], as illustrated in Figure 2. The resident stem cell lineages in the SGZ include the relatively quiescent radial NPC or type 1 progenitor cell population and the more rapidly dividing nonradial NPC or type 2 progenitor cell population. The lineage relationship between type 1 and 2 progenitors is unclear, but they ultimately give rise to migratory neuroblasts. As in the SVZ, the quiescent type 1 progenitors have a radial process that spans the granule cell layer and their cell bodies are in close contact with both their lineage-related progenitors and with blood vessels.

Endothelial cells

A major contributor in the NSC niche is the vasculature. The relationship between the vasculature and NPCs is not unique to the NSC niche. Vasculature also plays a directive role in the spermatogonia niche [30], the cancer stem cell niche [31] and during organogenesis [32]. During neurogenesis, proliferative clusters consisting of NPCs, neuroblasts, glia and endothelial cell precursors are found associated with small capillaries in the hippocampus [33]. Similar associations are seen in the lateral ventricle [34]. This association is also significant in pathological conditions such as stroke, where responding NPCs set up atypical vascular niches that enhance their own proliferation and differentiation [35,36]. When ECs of varying origin and NPCs are cocultured *in vitro*, ECs promote NPC self-renewal, suppress differentiation and, when differentiation does occur, promote neural differentiation [37]. The signals mediating these events are most likely a combination of common mitogens and cooperative signaling networks. Many molecules initially appreciated to have vascular effects also affect the neural system and *vice versa* [38–41]. Vessels also provide a specialized basal lamina that is critical for their role as a niche component [42]. Recent evidence suggests that basal laminar fractones play a role in sequestration of NPCs in the niche. Blockage of the laminin $\alpha 6 \beta 1$ receptor expressed by SVZ progenitor cells *in vivo* led to the release of cells from the basal lamina and a subsequent increase in proliferation [26]. This increase in proliferation is likely indicative of a shift in the lineage of the cells from the quiescent type B cells to proliferating type C cells. In fact, further differentiated type A cells downregulate expression of the laminin receptor. This understanding of vascular-derived factors mediating niche residency could be very useful for the *ex vivo* expansion of exogenous NPCs and as a potential target for mobilization of endogenous NPCs.

Engineering the neurovascular niche provides constructs that are useful for both neurogenic and angiogenic applications. In a 2D EC and NPC coculture model of the neurovascular niche, NPCs promote EC tube formation and stabilization *in vitro*, while ECs promote NPC proliferation [43]. When this coculture model was translated to 3D using a poly(ethylene glycol)/poly(L-lysine) (PEG/PLL) macroporous hydrogel, NPC-mediated vessel stabilization was also seen in an *in vivo* subcutaneous implant model [44]. 3D *in vitro* modeling of NPC–brain endothelial cell interactions will be an invaluable tool to realistically study the molecular

signaling underlying these behavioral effects. In addition to these physiological studies, NPC–brain endothelial cell niche interactions have been used in tissue engineer constructs for spinal cord injury, stroke and transient ischemia. In a hemisection spinal cord injury model, implantation of rat brain ECs and postnatal rat NPCs on a two-component biodegradable scaffold promoted angiogenesis and blood–spinal cord barrier formation, with most NPCs remaining undifferentiated [45]. In a recent stroke study, ischemia-induced cortical NPCs were transplanted into the damaged brain with or without ECs [46]. The presence of ECs increased the survival, proliferation and neuronal differentiation of the grafted NPCs and lead to mild improvements in cortical function. Conversely, the ability of NPCs to modulate ECs has also been exploited in a mouse model of transient focal ischemia where embryonic NPC transplantation into an adult prior to induction of ischemia resulted in an increase in microvascular density [47]. The same supportive postischemic relationship has also been shown *in vitro* using adult NPCs [36].

The current data (see Table 1) demonstrate that exogenous NPCs can be used for the *in vivo* modulation of host or graft vasculature and that exogenous ECs can be used to modulate graft NPCs. The addition of ECs provides a potential solution to the problem of controlling NPC transplantation outcomes. However, despite the improvements in cellular behavior, the data supporting functional recovery are lacking. More work should be done in this field to determine optimal growth conditions that will maximize the benefits of these systems.

Astrocytes

Several different populations of astrocytes are present in the adult stem cell niche, including the putative NPC [48,49]. Other astrocytes within the niche participate in controlling NPC behavior. While both populations of astrocytes are morphologically similar, they express distinct markers, with NPCs expressing nestin but not s-100 β and niche astrocytes expressing s-100 β but not nestin [29,50,51]. Several factors expressed by niche astrocytes affect NPC behavior in physiological and pathological conditions [52–54]. These behavioral changes were demonstrated by Lim *et al.* in an *in vitro* culture of postnatal or adult-dissociated SVZ cells on an astrocyte monolayer [55]. Culturing the SVZ cells on the astrocyte monolayer promoted rapid proliferation of SVZ precursors and their differentiation into neuroblasts; this occurred in the absence of exogenous growth factors, which are normally a requirement for *in vitro* NPC proliferation. Specific populations of astrocytes are also capable of differentiating neuronal precursors to a particular type of neuron. A fetal mid-brain astrocyte feeder layer directed over 60% of human embryonic stem cells to a dopaminergic neuronal fate [56]. This behavioral control also occurs *in vivo* where transplantation of SGZ astrocytes or supplementation with the factors they produce is sufficient to induce neurogenesis in the normally non-neurogenic neocortex of adult mice [57].

Early efforts at engineering the astrocyte–NPC niche interaction have focused on cocultures of NPCs and astrocytes. In a 2D coculture of adult astrocytes and adult NPCs, hippocampal astrocytes but not spinal cord astrocytes promoted NPC proliferation and neuronal differentiation [58]. Not surprisingly, neonatal hippocampal astrocytes were more potent than their adult counterparts at mediating this effect.

These data suggest astrocytes as an important regional determinant of NPC niche localization. The ability of astrocytes to drive NPCs down a neuronal differentiation pathway has recently been applied in efforts to optimize guided nerve regeneration scaffolds. Adult hippocampal NPCs were grown on a laminin-coated micropatterned polymer substrate in the presence or absence of postnatal cortical astrocytes [59]. NPCs cocultured with astrocytes demonstrated increased neuronal morphology and an almost twofold increase in β -III tubulin expression. This differentiation was mediated by soluble cues from the astrocytes, with physical cell contact

partially suppressing the effect [60]. While these *in vitro* strategies are promising, NPC transplantation research may indirectly provided support for the utility of this approach *in vivo*. Previously, stimulation of endogenous NPCs and the supply of exogenous NPCs were understood to be two separate goals. However, in a recent Parkinson's model, transplantation of postnatal NPCs into the substantia nigra and striatum increased endogenous NPC proliferation, neuronal differentiation and migration to the injury site [61]. Notably, approximately 50% of the transplanted cells differentiated into astrocytes that expressed various neurotrophic factors including Sonic hedgehog, which is crucial for the function of niche astrocytes [57]. While these results are intriguing, the endogenous NPC activation could also be attributable to non-niche-associated factors expressed by differentiated astrocytes or to the other transplanted cells that did not differentiate into astrocytes.

The data outlined in this article (see Table 1) suggest a significant role for astrocytes in attempts to engineer the NPC niche. It is quite striking that transplantation of niche astrocytes is sufficient to change the microenvironment of non-neurogenic regions of the CNS. While astrocytes will certainly be useful as an assistant in the *ex vivo* expansion and differentiation of NPCs, given the gliosis that occurs secondary to many types of neural tissue damage, it is unlikely that these cells will be included as a component for *in vivo* transplantation. In addition, given that many of the desired effects can be achieved with soluble factors, it is unclear that transplantation would be necessary.

Ependymal cells

Ependymal cells are specialized glia that line the ventricular system of the brain and the spinal cord and produce cerebrospinal fluid (CSF). A layer of cilia covering the apical surface of these cells circulates the CSF, while their microvilli absorb CSF. Mirzadeh *et al.* utilized mouse SVZ whole mounts and confocal microscopy to look at the architecture of the ventricular surface [25]. At areas of adult neurogenesis, the ependymal cell layer is organized into pinwheel structures containing the apical ending of a NSC at the core and ependymal cells at the periphery. Ependymal cells were originally thought to be the resident stem cell population in the SVZ, but current evidence suggests that in the adult mouse brain these cells are postmitotic [62] and do not possess the ability to self-renew or differentiate into neurons in culture [63]. A recent paper from Carlen *et al.* confirms that ependymal cells do not play a role in adult neurogenesis under normal conditions, but demonstrates their ability to differentiate into neuroblasts and astrocytes in response to stroke [64]. However, even under these circumstances they do not possess all of the characteristics of stem cells and become depleted owing to their inability to self-renew.

While they are probably not NSCs under normal conditions, ependymal cells do play a role within the stem cell niche. When looking at ependymal and subependymal cell cocultures in an effort to identify which were NPCs, Chiasson *et al.* demonstrated an increase in the EGF- or FGF-mediated proliferation of the NPC subependymal population [63]. Since this study, several factors released by ependymal cells have been identified that affect NPC behavior. Ependymal cells, along with vascular cells, release pigment epithelium-derived factor, which promotes NPC self-renewal *in vitro* and *in vivo* [65]. Ependymal cells also release noggin, a bone morphogenetic protein (BMP) antagonist [66]. BMP, which is produced by NPCs and transit amplifying cells, normally prevents neurogenesis by directing cells to a glial fate. In addition, ependymal cells play a role in migration of newly born neuroblasts leaving the niche. The flow created by the cilia of ependymal cells is required to generate gradients of chemorepulsive slit proteins produced by the septum and choroid plexus [67]. Engineering efforts in this area have not been directly pursued, but given the recent advances in understanding the signals ependymal cells produce this will likely be a growing field in the future.

Extracellular matrix

The majority of work in engineering the neural stem niche has focused on mimicking various chemical and mechanical properties of the niche ECM. The native ECM of the NPC niche consists of a 3D scaffold with incorporated signaling moieties and bound growth factors and cytokines, which are produced by the cellular components embedded in the ECM. These signaling moieties and bound factors, along with the architecture and mechanical properties of the ECM, dictate cell behavior. Like the ECM in the rest of the CNS, NPC niche ECM is largely composed of hyaluronic acid (HA) and its associated glycoproteins and proteoglycans (PGs) [68]. Rodent fetal and postnatal NPCs produce multiple chondroitin sulfate PGs (CSPGs) that bind HA [69,70]. Astrocytes are also an important source of CSPGs, as well as growth factors. *In vitro* these CSPGs augment FGF-2 mediated proliferation and impair neuronal differentiation; however, once differentiated, NPCs downregulate CSPG expression [69–71]. In addition, the ECM surrounding the niche contains tenascin C, fibronectin, laminin, thrombospondin and collagen IV [28,72,73]. Tenascin C is highly expressed in the SVZ, and tenascin C-deficient mice have altered numbers of NPCs with an increased probability of generating neurons in culture [74]. The expression of laminin, fibronectin and their receptors vary throughout development. Notably the expression of the laminin $\alpha 2$ chain gradually becomes restricted to the SVZ, while laminin 1 is enriched in blood vessels throughout development [75].

Natural and synthetic materials presented alone or with conjugated ligands and growth factors have been utilized to mimic the behavioral effects of the ECM on macro-, micro- and nano-scales. While natural materials already contain many bioactive elements, they are difficult to purify reproducibly. Synthetic materials allow for predictable control of chemical and mechanical properties, but lack the bioactive elements that play a critical role in instructing NPCs. To combine the best attributes of each material, synthetic materials have been modified to include bioactive elements [76] and most of the papers cited here use this approach. By altering the scaffolding material one can control the behavior of exogenous stem cells or guide the behavior of endogenous ones. Given the changes that occur in the ECM with aging and disease, this guidance will be especially important in the target patient population for regenerative strategies. In addition, the efficacy of artificial ECM constructs for *in vitro* cell culture and *ex vivo* cellular expansion platforms has been improved by the use of high-throughput techniques to investigate different combinations of factors present in the niche.

Engineering architectural cues

The architecture of the CNS ECM can be described as a HA meshwork with CSPG linkers anchoring the cell surface to the matrix scaffold. These CSPG linkages can either be direct or via complexes that associate with integrins and other cell surface receptors. Scaffold architecture also plays a role in stem cell response [77,78], and mimicking the scaffolding provided by the ECM has proven beneficial during *in vivo* stem cell transplantation. In a rat transection spinal cord model, a self-assembling peptide nanofiber scaffold consisting of interwoven nanofibers (~10 nm diameter) was used for cell transplantation. Use of this L-amino acid-based scaffold improved the organization and integration of transplanted NPCs [79,80]. Christopherson *et al.* recently studied the effect of varying scaffold fiber diameter on adult rat hippocampal NPC behavior using a laminin-coated electrospun poly(ethersulfone) mesh [81]. Smaller diameter fibers (283 nm) promoted proliferation and differentiation to oligodendrocytes, while midsize fibers (749–1452 nm) promoted neuronal differentiation. These studies (see Table 2) suggest that scaffold architecture is an ECM property that can be mimicked to improve transplantation outcome and can be varied to direct NPC differentiation down a desired lineage. While there are undoubtedly many other factors that serve as *in vivo* determinants for NPC fate, scaffold architecture is one that can be cleanly and consistently

controlled in an *ex vivo* expansion application. Furthermore, implantation of NPCs on a scaffold that promotes a desired cell fate could be used to bias the fate of cells *in vivo*.

Engineering mechanical cues

In addition to the architecture of the scaffolding, cells are responsive to its mechanical properties, which can modulate morphology, proliferation and, in the case of stem cells, differentiation [82]. When mesenchymal stem cells are grown on soft substrates (0.1–1 kPa) they exhibit neuronal differentiation, stiffer matrices (8–17 kPa) promote myogenic differentiation and yet stiffer matrices (25–40 kPa) lead to osteogenic differentiation [83]. Mechanically, brain tissue from various species measured *in vivo* and *in vitro* is in the order of 1 kPa with some variation based on age and anatomical location [82,84–86]. To systematically study these effects, NPCs have been grown on materials whose elasticity can be easily modulated. Postnatal mouse NPCs seeded onto a library of mechanically and chemically tunable PEG/PLL hydrogels demonstrated increased migration on gels with elastic moduli on the order of brain tissue (3843–5316 Pa) and limited migration on gels of higher elastic moduli [87]. Subsets of gels with varying chemical properties also modulated stem cell differentiation. Similar results were seen using adult rat NPCs on a variable moduli interpenetrating polymer network, a tunable poly(acrylamide)-based hydrogel with modifiable surface chemistry [88]. NPCs seeded on RGD-modified variable moduli interpenetrating polymer networks with moduli more than 100 Pa proliferated in an undifferentiated state with maximal proliferation at moduli from 1–4 kPa. Softer substrates (100–500 kPa) promoted neuronal differentiation, while stiffer gels (1–10 kPa) promoted glial differentiation. Both studies, which are summarized in Table 2, confirm that recapitulating NPC *in vivo* behavior is facilitated by the use of a substrate with an elastic modulus in the order of brain tissue. Given the changes in ECM modulus that are seen with aging and disease, it may be necessary to transplant NPCs on a scaffold with a younger or more physiologic elastic modulus.

Engineering bioactive cues

Bioactive polymers have also been used as a scaffolding material for NPCs (see Table 2). These polymers contain conjugated signaling molecules or peptide sequences that are either contained within the ECM or presented by the ECM after being produced by other cellular niche components. Peptide sequences are often used in place of full protein conjugates owing to the simplified conjugation chemistry and lower cost. One of the first bioactive polymer scaffolds presented the laminin-1-derived IKVAV peptide sequence, which was previously known to promote neurite outgrowth and sprouting, at a superphysiologic density [89]. When fetal NPCs are encapsulated in this self-assembling nanofiber scaffold they rapidly differentiate into neurons and astrocytic differentiation is suppressed. *In vivo* injection of the scaffold in a clip compression spinal cord injury model reduces cell death and gliosis while increasing oligodendroglia, fiber regeneration and behavioral recovery [90]. Another self-assembling peptide construct, the RADA 16 scaffold, has been used to investigate functional motifs from laminin, collagen VI, fibronectin and bone marrow-homing molecules [91]. Growth of adult NPCs on the amphiphilic RADA16 scaffold increased the percentage of undifferentiated nestin-positive cells when compared with Matrigel™, while the addition of bone marrow-homing sequences promoted neural differentiation.

In addition to studying individual motifs, combinatorial studies have been undertaken. In a study investigating peptide combinations and densities, interpenetrating polymer network hydrogels with acrylamide, PEG 1000 mono-methyl ether monomethacrylate and acrylic acid monomers were modified with either bone sialoprotein-derived RGD or IKVAV [92]. While the RGD motif promoted cell attachment, enhanced self-renewal and increased media-induced differentiation of adult NPCs in a dose-dependent manner, the IKVAV motif did not promote

cell attachment and had no effect on self-renewal or differentiation. These differences in results with the IKVAV motif may be due to the different cell types used (adult NPCs vs fetal NPCs or mature neurons) or the density of peptide presentation. Even with the use of fetal NPCs, a lower density of IKVAV is not sufficient to induce neuronal differentiation [89]. These distinctions highlight the complexity of mimicking ECM bioactive motifs. In addition to the manner of presentation, there are also various isoforms and numerous post-translational modifications that can affect behavioral outcome.

Engineering growth factor cues

The commonly recognized growth factors EGF and FGF are essential niche components that are routinely used to culture NPCs *in vitro* [93–96]. Within the niche, neurogenesis tends to occur around the laminin-positive fractones, which bind and concentrate FGF-2 [26,28]. Previously, interaction of individual growth factors with NSCs has been studied by immobilizing growth factors on scaffolds or by assembling cells around growth factor-releasing microparticles [97]. Recent efforts to understand how these growth factors affect interaction with other ECM components have utilized high-throughput screening arrays (see Table 2). A screening array with combinations of covalently linked ECM components and growth factors explored proliferation and differentiation of rat striatal NPCs [98]. While the predominant effects in this study were due to the growth factors, the type of ECM component used was able to modulate the growth factor effect.

To investigate the impact of extracellular signaling on differentiation, bipotential human neural precursors were cultured on microenvironment arrays containing mixtures of ECM components, morphogens and other signaling proteins [99]. The authors were then able to classify the 44 combinations studied as neural promoting, glial promoting, neural and glial promoting, or neither neural nor glial promoting. Notably, cells costimulated with Wnt and Notch remained in a proliferative undifferentiated state. BMP-4, however, kept cells in an intermediate state expressing both glial and neuronal markers. This study demonstrates that in addition to recapitulating *in vivo* states, engineering platforms can be used to create novel states that could either reflect undiscovered *in vivo* scenarios or serve as useful artificial states.

Conclusion

While the role of the microenvironment in controlling NPC behavior was initially underappreciated, evidence now suggests that the niche is indispensable. Even the relatively simple act of growing NPCs in tissue culture requires growth factors that are presented by the niche *in vivo*. Mimicking elements of the NPC niche has proven to be a promising direction for controlling NPC behavior for regenerative medicine applications. In particular, *in vivo* strategies recapitulating aspects of the NPC niche ECM have proven useful in therapeutically guiding the fate of NPCs. While use of cellular niche elements is a relatively younger field, it will provide a nice complement to the ECM studies. The addition of cellular components will allow for the provision of very complex signals at physiologically relevant doses and timings, and the ability for the system to respond to physiological stressors.

Future perspective

Future efforts to engineer the NPC niche will need to use combinatorial approaches to mimic multiple aspects of the niche environment. For *in vitro* tissue culture and *ex vivo* expansion applications, this will most likely consist of a designer ECM scaffold seeded with NPCs and a second or even third cellular niche component. For *in vivo* tissue engineering constructs, this will likely consist of a designer ECM scaffold seeded with NPCs that have been primed with other niche components or soluble niche factors. Given that the first human NSC transplantation study has shown no ill effects in its Phase I trial, and that the first embryonic

stem cell clinical trial for spinal cord injury was recently approved, the ability to improve the survival and direct the differentiation of implanted CNS stem cells will be a critical next step.

Executive summary

The neural stem cell niche

- Neural stem cell characteristics represent a state that a cell enters based on signals from the microenvironment.
- If neural progenitor cells (NPCs) are transplanted to a non-neurogenic region of the CNS, they no longer produce neurons. Conversely, if proliferative non-neurogenic cells are transplanted into a neural stem cell niche, they will self-renew and produce differentiated progeny.
- The NPC niches, the subgranular zone and subventricular zone, consist of resident stem cells, endothelial cells, astrocytes, ependymal cells (in the subventricular zone) and the surrounding extracellular matrix (ECM).
- The ability to control the behavior of NPCs transplanted for regenerative therapy will require an engineered stem cell niche.

Extracellular matrix

- The ECM consists of a 3D scaffold with incorporated signaling moieties and bound growth factors and cytokines.
- ECM architecture, mechanics, signaling moieties and bound growth factors can all be utilized to control the behavior of NPCs.

Endothelial cells

- Neurogenesis and angiogenesis are linked through common signaling molecules and are integral in controlling one another in physiological and pathological circumstances.
- Exogenous implantation of endothelial cells can be used to modify graft NPC behavior and exogenous implantation of NPCs can be used to modify host or graft endothelial cell behavior.

Astrocytes

- Niche astrocytes promote NPC proliferation and neurogenesis while astrocytes from non-neurogenic regions do not.
- Given the extensive gliosis present in a wide variety of CNS injuries, astrocytes will most likely be used as *in vitro* tools, while the soluble factors they secrete may be most useful *in vivo*.

Ependymal cells

- While ependymal cells do not normally function as stem cells, under pathological conditions they can be induced to undergo neurogenesis.
- While ependymal cells have yet to be used in engineering the NPC niche, they have been shown to control NPC behavior via the production of pigment epithelium-derived factor and noggin.

Future perspective

- Future efforts in engineering the NPC niche will likely be a combination of ECM-based scaffolds with NPCs, and the addition of other cellular niche components.

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Figure 1. Subventricular zone niche

The subventricular zone contains neural progenitor cells (type B cells), which are morphologically similar to astrocytes and contact the ventricle through apical processes and blood vessels through basal processes. The apical process of the type B cells is surrounded by ependymal cells, while the bodies of the type B cells support chains of migrating neuroblast (type A) cells. Along these chains of type A cells reside clusters of proliferating transit-amplifying cells (type C cells). Type B cells can either self-renew or give rise to type C cells, which in turn produce type A cells. Vessels in the niche produce a laminin-rich extravascular basal lamina, which contacts other cells in the niche and is organized into branched structures known as fractones.

Figure 2. Subgranular zone niche

The subgranular zone contains relatively quiescent radial neural progenitor cells (type 1 progenitor) whose radial processes span the granule cell layer. The more rapidly dividing nonradial neural progenitor cells (type 2 progenitor) are in close contact with the cell bodies and processes of the type 1 progenitors. These progenitors give rise to neuroblasts and, ultimately, granule neurons.

Table 1
Overview of studies using cellular components to engineer the CNS niche

Cells	Scaffold	Model	Outcome	Ref.
Endothelial cells	Brain-derived ECs; postnatal NPCs	N/A	Increased NPC proliferation	[43]
	Brain-derived ECs; postnatal NPCs	Subcutaneous implantation	Increased implant vessel stability	[44]
	Fat pad-derived ECs; postnatal NPCs	PLGA 405 oriented outer plus PEG/PLL macroporous inner	Increased implant vessel density; increased BSB formation	[45]
	Adult cortical-derived ischemia-induced NPCs; bovine pulmonary micro-vascular ECs	N/A	Increased graft NPC survival, proliferation and neuronal differentiation; mild improvements cortical function	[46]
	Embryonic NPCs	MCAO ischemia	Increased endogenous microvascular density	[47]
Astrocytes	Adult NPCs; adult hippocampal astrocytes	N/A	Increased NPC proliferation and neuronal differentiation	[57]
	Adult NPCs; postnatal cortical astrocytes	Laminin-coated micropatterned polystyrene	Increased neuronal differentiation	[58,59]
	Postnatal NPCs	N/A	Increased host NPC proliferation, neuronal differentiation and migration to injury site; ~50% of graft NPCs differentiated into Shh-expressing astrocytes	[60]

BSB: Blood-spinal cord barrier; EC: Endothelial cell; N/A: Not applicable; MCA: Middle cerebral artery; MCAO: Middle cerebral artery occlusion; NPC: Neural progenitor cell; PEG: Poly (ethylene glycol); PLGA: Poly(lactic-co-glycolic acid); PLL: Poly(L-lysine); Shh: Sonic hedgehog.

Table 2
Overview of studies using extracellular matrix components to engineer the CNS niche

Cells	Scaffold	Model	Outcome	Ref.
Architectural cues	Adult NPC	Self-assembling peptide nanofiber	Transsection spinal cord injury	Improved organization, integration of transplanted NPCs [79,80]
	Adult NPCs	Laminin-coated electrospun PES mesh, varying diameter	N/A	Small diameter = increased proliferation and oligodendrocyte differentiation Medium diameter = neuronal differentiation [81]
Mechanical cues	Postnatal NPCs	PEG/PLL hydrogel	N/A	Increased migration on gels with elastic modulus similar to brain tissue [87]
	Adult NPCs	Polyacrylamide moduli interpenetrating polymer network	N/A	Increased proliferation with elastic modulus similar to brain tissue; increased neuronal differentiation on softer gels; increased glial differentiation on harder gels [88]
Bioactive cues	Embryonic NPCs	Self-assembling peptide IKVAV	Clip compression spinal cord injury	Decreased cell death and gliosis; increased oligodendroglia, fiber regeneration and behavioral recovery [89,90]
	Adult NPCs	Self-assembling peptide RADAI6	N/A	Decreased NPC differentiation [91]
	Adult NPCs	Acrylamide/PEG/acrylic acid interpenetrating polymer network	N/A	RGD increased cell attachment, enhanced self-renewal and media-induced differentiation of adult NPCs [92]
Growth factor cues	Bipotential NPCs	Microenvironment arrays with mixtures of ECM components, morphogens and other signaling proteins	N/A	Classified 44 combinations as neural promoting, glia promoting, neural and glial promoting, or neither neural nor glial promoting [99]

ECM: Extracellular matrix; N/A: Not applicable; NPC: Neural progenitor cell; PEG: Poly (ethylene glycol); PES: Polyethersulfone; PLL: Poly(L-lysine).